Harvard-MIT Division of Health Sciences and Technology
HST.535: Principles and Practice of Tissue Engineering
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GENERAL HOSPITAL OF PLA

LIVER CELLS

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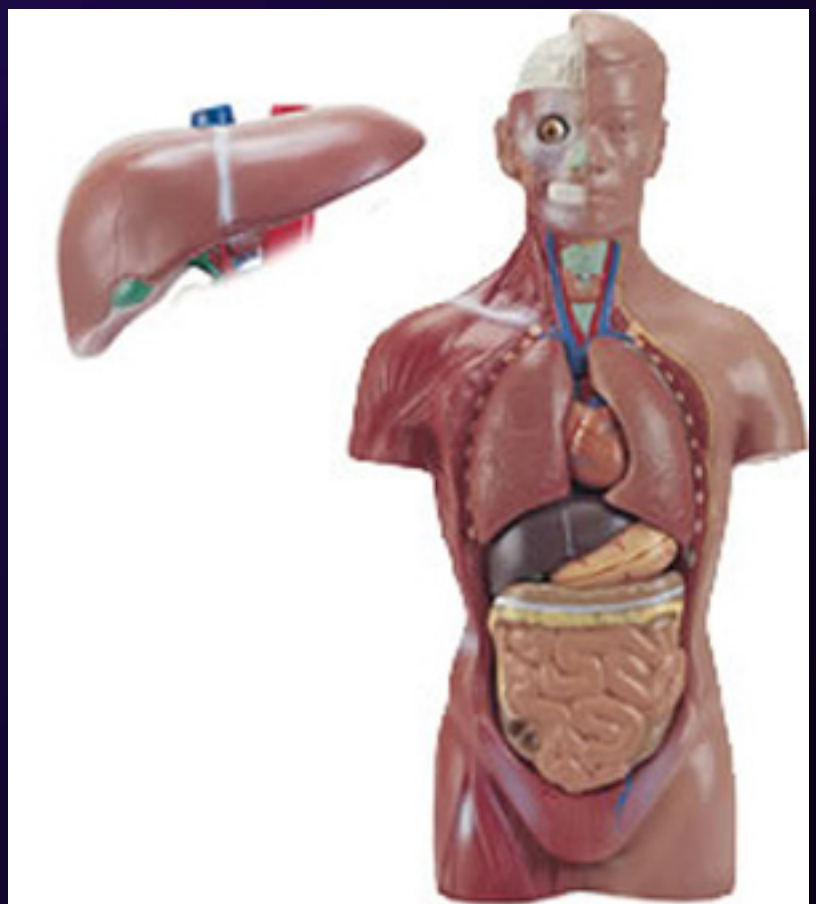
Liver Cells

- Structure and function of Liver
- Regenration of liver cells
- Liver cells research relevant to liver tissue engineering

Liver: the largest compound gland and chief metabolic organ



Courtesy of US Dept. of Health and Human Services.

Different Types of Liver Cells

- Hepatocytes (parenchymal cells,PC)
- Liver endothelial cells (LEC)
- Kupffer cells (KC)
- Stellate cells(SC)
- Other cells:
  - epithelial cells of bile duct
  - endothelial cells of blood and lymphatic vessels
  - smooth muscle cells of arteries and veins
  - nerve cells
  - fibroblasts
  - inflammatory cells

Arrangement of liver cells

Two diagrams of liver structure removed for copyright reasons.

Source: Cormack, *Clinically Integrated Histology*.

Histological structure of liver

Photos removed for copyright reasons.

Fig.1: The direction of blood flow (arrow) from the branch of the portal vein (V) toward sinusoids (S) in the liver , (D) bile duct, (A) branch of the hepatic artery. ×344

Fig.2: The direction of blood flow (arrow) from sinusoids (S) to the central vein (V) of the liver. × 140

Fig.3: A sinusoid (arrow) emptying into the central vein (V) of the liver. × 344

Irwin Berman,
Color Atlas of Basic Histology

Functions of liver cells

- Intricately involved in carbohydrate, fat, and protein metabolism.
- Store vitamins and minerals; form specific compounds such as coagulation factors and somatomedins or growth factors.
- Filter the blood, removing organic by-products, cellular debris, and many other particles.
- Produce and secrete bile.
- Detoxifie or excrete cholesterol, steroid hormones, drugs, pesticides, and other toxic compounds

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Liver Regeneration

Prometheus alleged phenomenal powers of liver regeneration are enshrined in Greek mythology

The most widely studied model of liver regeneration is the rat liver after two-thirds partial hepatectomy (PH), involving removal of the median (M) and left lateral (LL) lobes

.

regeneration in the residual lobes restores preoperative liver mass within a few days.

Malcolm R. Alison
CELL & DEVELOPMENTAL BIOLOGY, vol13, 2002,385–387

Factors related to Liver regeneration

- Cell sources

  Hepatocytes, hepatic stem cells (oval cells) and bone marrow diliverstem cells

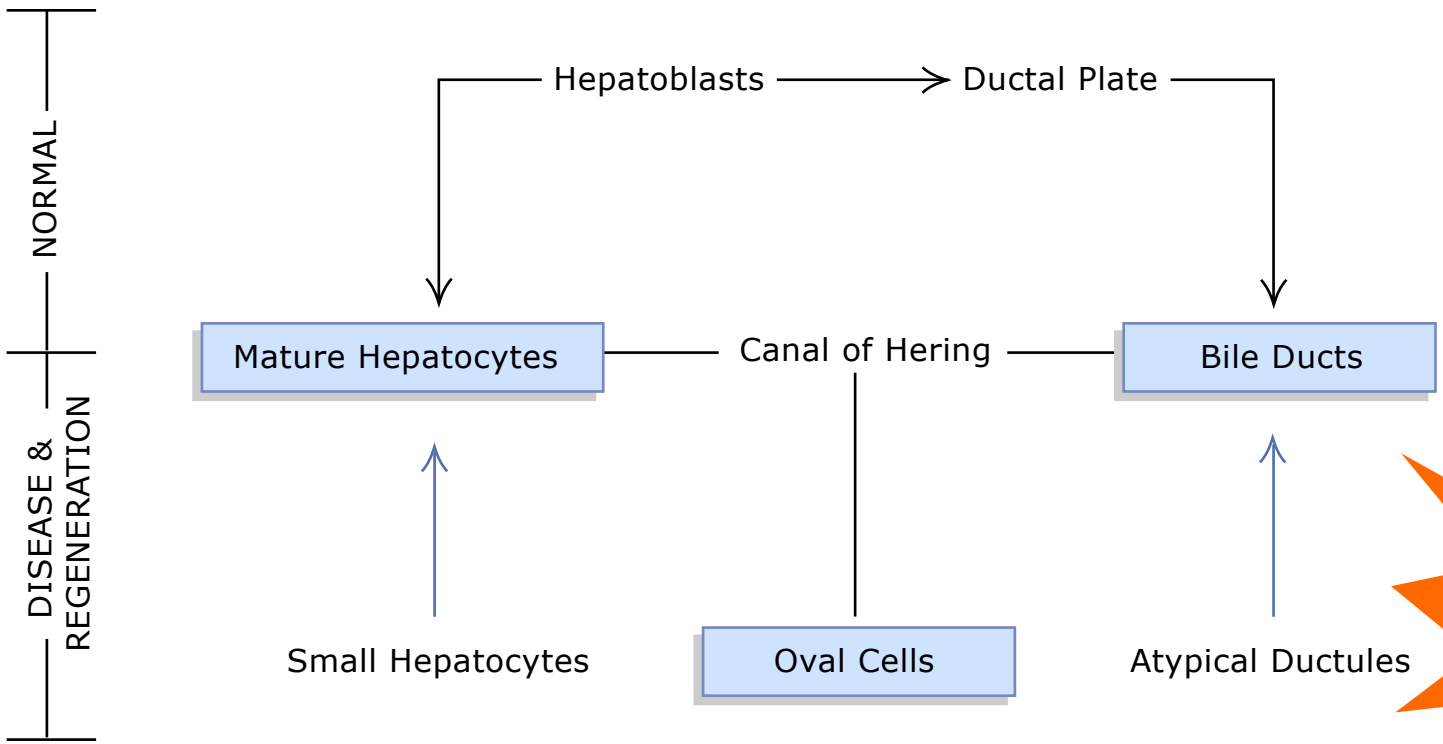
- Growth and regulating factors

  HGF, EGF, TGF-β,TNF-α, IL-6, IL-1, VEGF…

- Influences of non-parenchymal cells

  Stellate cells, Kupfer cells, endothelial cells

Liver regeneration during injury



The blue boxes: bone marrow haemopoitic stem cells have been incorporated
On the top: normal fetal liver development
On the bottom: disease and regeneration

Figure by MIT OCW. After Crosby et al., *Cell and Developmental Biology.*

H.A. Crosby et al.
CELL & DEVELOPMENTAL BIOLOGY, Vol. 13, 2002: pp. 397–403

Interactions between cells during regeneration

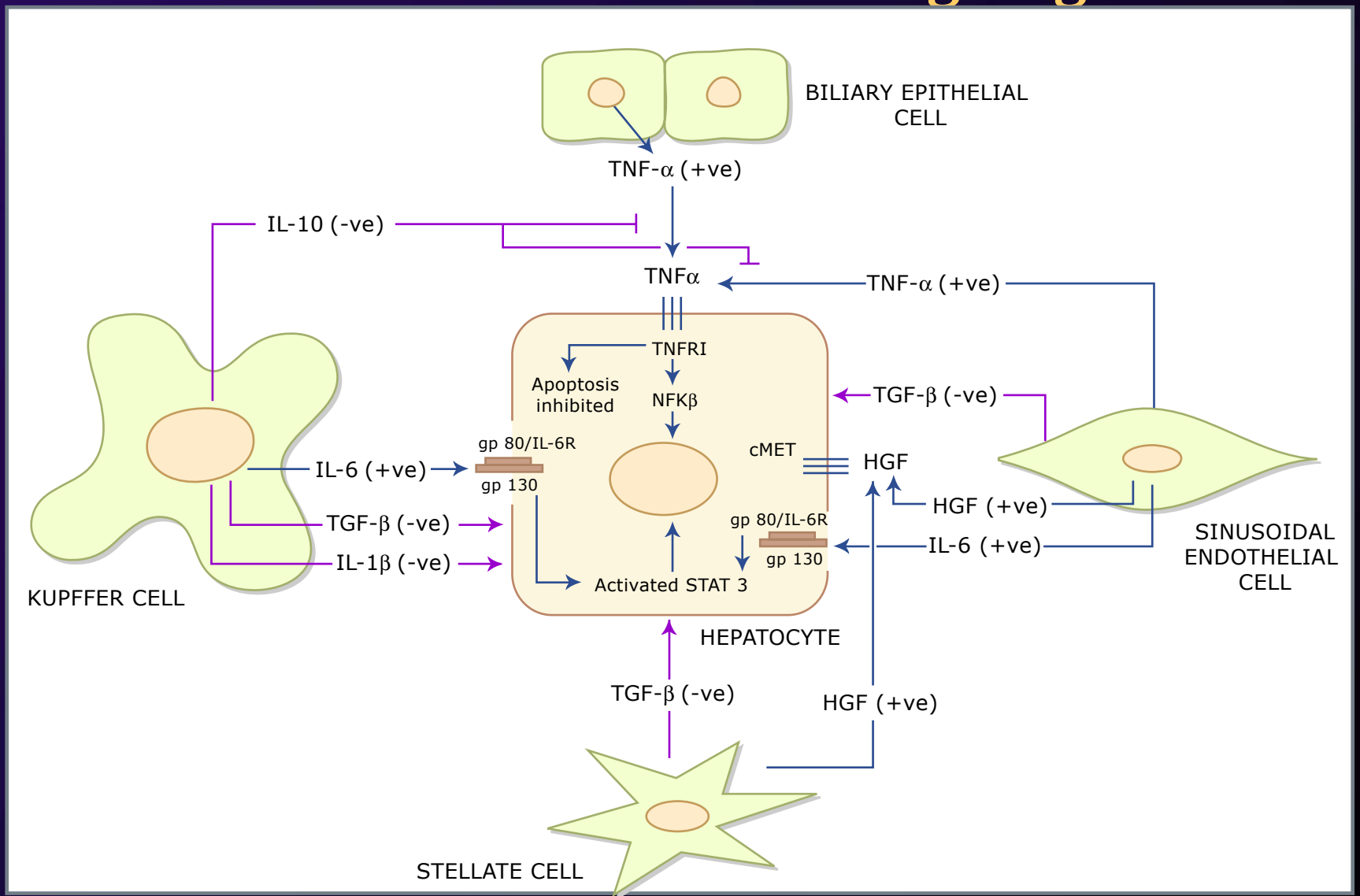


Figure by MIT OCW.

R. Malik et al. CELL & DEVELOPMENTAL BIOLOGY, Vol. 13, 2002: pp. 425–431

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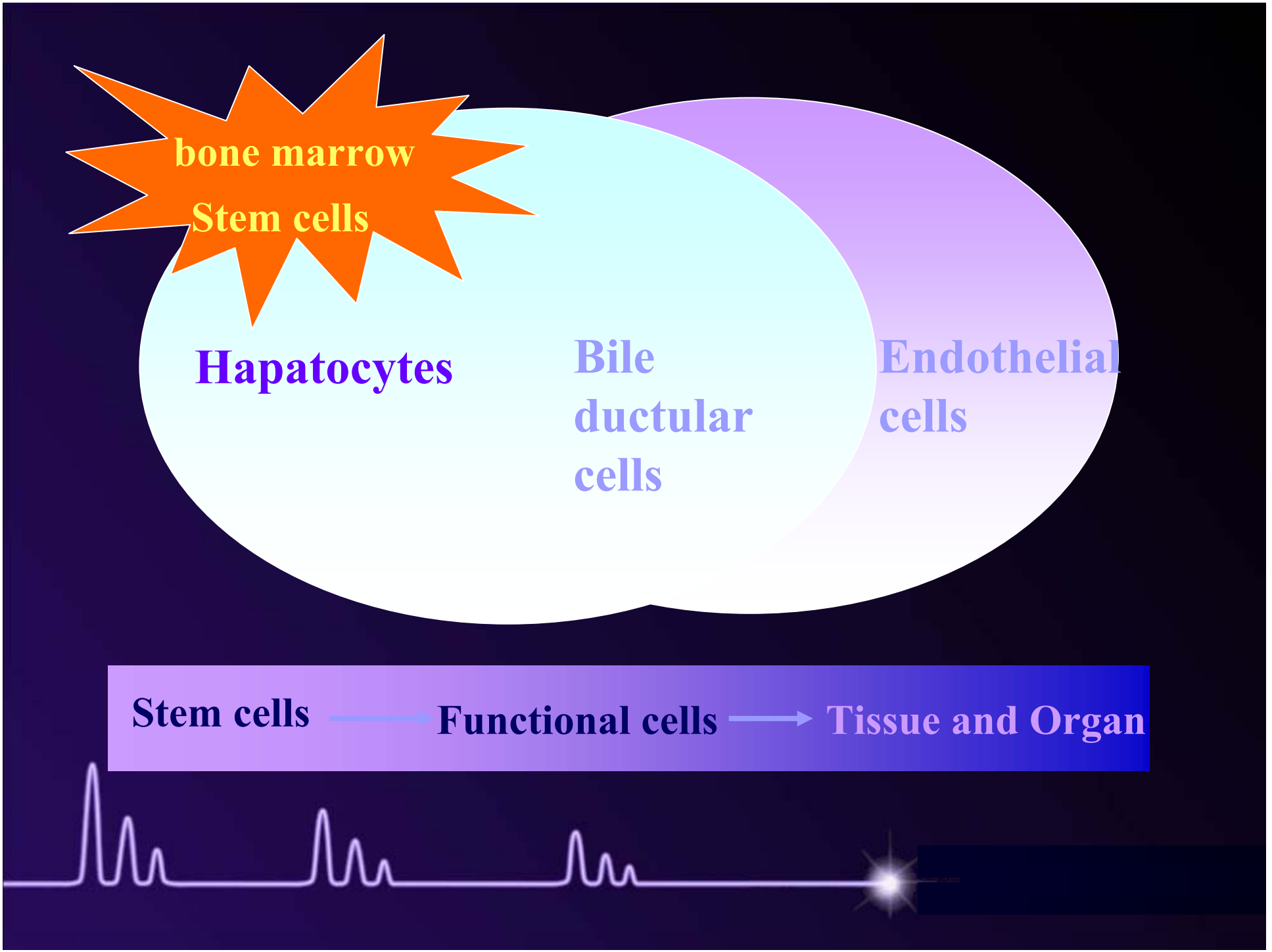
Liver Tissue Engineering

- Cell sources
- Compatibility of materials to hepatocytes
- Cytological research related to tissue vascularization

Hepatocyte sources

- Primary hepatocytes
- Tumour-derived cell lines HepG2 and C3A
- Embryonic stem cells
- Adult stem cells
  - small hepatocyte
  - oval cell
  - bone marrow derived stem cell


bone marrow
Stem cells
Hapatocytes
Bile
ductular
cells
Endothelial
cells
Stem cells
Functional cells
Tissue and Organ

From BMSCs to Hepatocytes

- Culture medium: DMEM, IMDM,
- Growth Factors: HGF, EGF and extraction of regenerative liver tissue
- ECM: Collagen coating , Poly-Lysine coating
- Identify methods:
  - Morphology observation
  - Immunofluorescence(Albumin, CK8, CK18),
  - RT-PCR (Albumin)
  - Radioimmunon analysis (AFP)

From Bone Marrow Cells to Hepatocytes

Photos removed for copyright reasons.

BMSCs in IMDM **BMSCs+HGF+EGF**

Photos removed for copyright reasons.

CK18 **Albumin**

From BMSCs to Hepatocytes

Effect of Partial Hepatectomy Experiment I:

- Animal : Kunming mouse

  Group A: sham operation (n=20)

  Group B: partial hepatectomy (2/3) (n=20)

- BMSCs isolation

  At 12h, 24h, 36h 48h, 72h after operation respectively

- BMSCs culture

  BMSCs were cultured in IMDM +HGF + EGF

- Immunoflurescence stain

  Counting the ALB positive cells and calculating the differentiation rate

ALB positive rate:

At 24h following operation:

Group A: 10.43 %, Group B: 9.83 % ($P<0.05$)

From Bone Marrow Cells to Hepatocytes

Albumin staining

Photos removed for copyright reasons.

CK 18 staining

sham operation (24h) partial hepatectomy (24h)

From BMSCs to Hepatocytes

Effect of Partial Hepatectomy Experiment II:

- Animal : Kunming mouse, partial hepatectomy (PH 2/3)
- Liver tissue lixivium (LTL)

  Regenerative liver tissue were extracted at 36h after PH
- BMSCs isolation and culture

  BMSCs + IMDM

  BMSCs + LTL

  BMSCs + IMDM +HGF + EGF

  BMSCs + IMDM +HGF + EGF+ LTL
- Immunoflurescence stain

  Counting the ALB positive cells and calculating the differentiation rate

From Bone Marrow Cells to Hepatocytes

BMSC labelled by BrdU

Photos removed for copyright reasons.

Photos removed for copyright reasons.

Distribution of induced cells labeled by Brud in liver fibrosis tissue (liver tissue section)

Induced cells labeled with BrdU(CLSM)

Red: albumin positive
Green: BrdU positive
Orange: albumin+BrdU positive

Endothelial Cells Source

- Primary endothelial cells
- Endothelial progenitor cells(EPCs)
- Embryonic stem cells
- Bone marrow derived stem cell

From Bone Marrow Cells to Endothelial cells

Photos removed for copyright reasons.

Rat BMSCs

At 14 day after induced

Photos removed for copyright reasons.

vWF-FITC(VEGF)
7day

FLK1(VEGFR-2)-TRITC
14 day

Liver Tissue Engineering

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Evaluating biocompatibility of scaffold materials

- Liver cells isolation and culture
- Contrast microscopy
- Scan electronic microscopy（SEM）
- Laser confocal microscan system (LSCM)
- Biochemical analysis of culture medium

Several slides removed for copyright reasons.

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Thank you!